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PLANT–MICROBE–INSECT INTERACTIONS

Plant feeding by insect vectors can affect life cycle, population genetics and evolution of plant viruses

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Summary

1. Transmission from host to host is a crucial step in the life cycle of pathogens, particularly of viruses, ensuring spread and maintenance in host populations. The immobile nature of plants and the strong pectin and cellulose barrier surrounding cells have constrained most plant virus species to use vectors (mainly insects) for exit, transfer and entry from one host to another.
2. A growing body of evidence is showing that plant viruses can influence vector physiology and behaviour to increase their chances of transmission, either directly or through modification of the host plant. In contrast, little is known on the possible reciprocal interaction, where the vector way of life would significantly impact on the viral behaviour and/or phenotype within the infected plants, on its population genetics and its evolution.
3. The complex possible reaches of these three-way interactions on the ecology of each partner have not been exhaustively explored.
4. After briefly summarizing the current knowledge on how viruses can induce changes in insect vector behaviour, physiology and population dynamics, this review focuses on presenting unforeseen aspects related to (i) the impacts that the feeding habits of different insect vectors can have on the evolution of plant viruses and (ii) the possibility that vector-related stresses induce major switches in the 'behaviour' of viruses *in planta*, affecting primarily the efficiency of transmission by insect vectors.

Key-words: insect, insect, plant stress, plant virus, population genetics, vector transmission, virulence, virus ecology, virus evolution

Introduction

The interplays between herbivorous insects, plants and microbes are impressively diversified and constitute a fascinating field of investigation, both for the richness of the underlying molecular and physiological mechanisms of attack, defence or mutualistic interaction and for the extension of these relationships in a much broader ecological context (Pieterse & Dicke 2007). When microbes are developing within plant hosts, they strive for the same resources as herbivorous insects and thus at first sight appear opposed in a competition. Whether these microbes have mutualistic or pathogenic interactions with the host plant, they might help defend against herbivores. A frequent sophistication in this simple scheme is when plant-to-plant transmission of microbes is mediated by vectoring herbivorous insects. This creates 'the inseparable ecological trinity' (Carter 1939), where microbes obviously compete with their vectors for the plant resource, but also depend on their presence for transmission. Hence, a dilemma can be expected, where microbes need the vectors but not at every phase of their life cycle. Vector transmission occurs in bacterial and fungal systems, but is particularly common in plant–virus systems. The present review will thus largely focus on viral pathogens. Based on known viral vector transmission examples, we will pin down where tripartite relationships between viruses, plants and insects could have unforeseen implications in a broader ecological context.

The transmission of plant viruses by insect vectors has been studied for over a century (Takami 1901). In the early era, studies addressed the output of the whole three-partner system sometimes even incorporating environmental factors. Countless publications quantified the transmission rate for a given plant–virus–vector system, the optimal acquisition time of the virus by the vector, the optimal

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inoculation time and the latent period required between acquisition and inoculation (for reviews, see Harris 1983; Nault 1997). It was early noted that these parameters were influenced by factors other than the simple nature of the three interacting species, such as the age of the host plant, the developmental stage of the vector or the effect of temperature (Anhalt & Almeida 2008 and references therein). Details of the underlying mechanisms were not accessible at that time, and this is certainly why these studies gave way to more explicative but more reductionist approaches, using for example electron microscopy and then molecular biology, as these technologies became accessible. Electron microscopy has been instrumental in elucidating the cycle of various viruses within the body of their insect vectors (Peiffer, Gildow & Gray 1997). Molecular biology has provided a wealth of knowledge on structures and functions of different viral genes and proteins, notably on genes involved in direct interactions with the insect vectors (Ziegler-Graff & Brault 2008). However, interesting and mandatory as they were, vector transmission–related molecular studies have nearly exclusively concentrated on the couple of virus–vector and ignored the plant for over three decades. The plant–insect and plant–virus research fields have also mostly developed as separate disciplines (Malmstrom, Melcher & Bosque-Perez 2011), with too few efforts to integrate information in the larger framework of plant–virus–insect interactions. While this integrative trend is now developing among the related scientific community (Malmstrom, Melcher & Bosque-Perez 2011), some relevant properties of viruses, plants and insect vectors are still to be envisioned as important players of a complex system, having potential ecological implications.

Several studies have proven that virus-induced plant reactions can influence the behaviour, the physiology and the dynamics of insect vectors in plant populations, sometimes provoking changes in the insect that are favourable to virus transmission (Bosque-Perez & Eigenbrode 2011). In contrast, a modification of the virus 'behaviour' within the host plant in response to attack by herbivorous insect vectors has not been addressed until very recently (Blanc, Uzest & Drucker 2011). In fact, perhaps because viruses are often seen as extremely simple biological entities, their capacity to actually react in an adapted way to the plant stress resulting from feeding by their insect vectors is still not envisaged. But if one transcends this dubious limitation of viruses, it is tempting to imagine that a virus could sense its vector 'signal' into the plant and behave accordingly to optimize transmission. According to this hypothesis, the viral reaction could be to make itself better accessible and/or to accumulate in the vector and kill the host plant to favour dispersal. Indeed, it is important to realize that the initial common fate of individual plants and an infecting virus becomes uncoupled when a vector is present (further discussed in the last section).

Another integrative aspect needing further attention lays in the impact of vector transmission on the evolution of viruses. Viral population genetics have tremendously developed during the last decades (Elena *et al.* 2011). Individual plants have been shown to represent heterogeneous 'landscapes' where the genetic diversity of virus populations is not distributed uniformly. Different vector species with different feeding habits could thus access distinct viral subpopulations within the host, and successive transmission cycles by one vector species, by another, or by alternation thereof could obviously change the viral evolution scenario. In turn, whether viruses have developed specific strategies where subpopulations accessible to the vector would be specifically differentiated is also an interesting aspect of the system that awaits investigation.

Hereafter, we briefly review information on plant–virus, plant–vector and virus–vector relationships that is relevant to understand the potential effects of vector feeding behaviour on plant virus evolution and emphasize the need for further integration of this knowledge into a picture combining all three partners and their environment. We will then consider the expected consequences of these interactions at the broader scale of epidemiology and ecology.

Virus–vector interactions

Three major modes of vector transmission have been described thus far (Fig. 1, Tables 1 and 2). The description of the corresponding virus–vector relationships in this section is mandatory for understanding and foreseeing the possible adaptive strategies of mutual manipulation between plant, virus and insect vector. Although the three transmission categories can to some extent accommodate viral transmission by any type of vector (Table 1), they

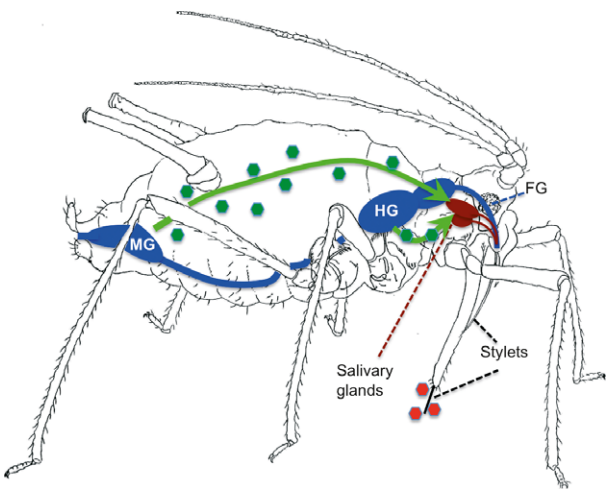

Fig. 1. Different routes of plant viruses in their aphid vector. Within an aphid, the gut is represented in blue and the salivary glands and salivary duct in brown. The green arrows represent the cycle of circulative viruses (green hexagons) within the aphid body, across the gut epithelium to the haemolymph and to the salivary glands. Non-circulative (non-persistent) viruses appear at their attachment sites at the tip of the stylets as red hexagons. FG, foregut; MG, midgut; HG, hindgut. The aphid drawing was kindly provided by N. Sauvion.

Table 1. Vectors and modes of transmission in families of plant viruses

Family*	Vector	Mode of vector transmission†
Bromoviridae genus *Alfamovirus*	Aphids	Non-circulative capsid strategy
Bromoviridae genus *Cucumovirus*	Aphids	Non-circulative capsid strategy
Bromoviridae genus *Ilarvirus*	Thrips	?
Bromoviridae genus *Oléavirus*	?	?
Bromoviridae genus *Bromovirus*	Beetle	?
Bunyaviridae	Thrips, planthopper	Circulative propagative
Caulimoviridae	Aphid, mealybug, leafhopper	Non-circulative helper strategy
Circoviridae	Aphid	Circulative non-propagative
Closteroviridae	Aphid, whitefly, mealybug	Non-circulative
Comoviridae genus *Comovirus*	Beetle	?
Comoviridae genus *Fabavirus*	Aphid	Non-circulative
Comoviridae genus *Nepovirus*	Nematode	Non-circulative capsid strategy
Geminiviridae	Leafhopper, whitefly	Circulative non-propagative‡
Luteoviridae	Aphid	Circulative non-propagative
Partitiviridae	?	?
Potyviridae genus *Potyvirus*	Aphid	Non-circulative helper strategy
Potyviridae genus *Ipomovirus*	Whitefly	Non-circulative
Potyviridae genus *Macluravirus*	Aphid	Non-circulative
Potyviridae genus *Rymovirus*	Mite	Non-circulative
Potyviridae genus *Tritimovirus*	Mite	Non-circulative
Potyviridae genus *Bymovirus*	Fungus	Circulative
Reoviridae	Planthopper, leafhopper	Circulative propagative
Rhabdoviridae	Leafhopper, aphid	Circulative propagative
Sequiviridae	Aphid, leafhopper	Non-circulative helper strategy
Tombusviridae	Fungus	Non-circulative

From reference Blanc (2008), with permission.
The non-circulative viruses, or assimilated as discussed in the text, are in blue. The circulative viruses, or assimilated as described in the text, are in green.
*The families are broken down to the genus level when they contain genera with totally different vectors and mode of transmission.
†The helper or capsid strategies (see Table 2) are mentioned when experimentally demonstrated for at least one of the member species. When no complement is added to either 'circulative' or 'non-circulative', it reflects the lack of further information.
‡For at least one member species [tomato yellow leaf curl virus (TYLCV)], replication within the vector is still being debated.
?No data is available.

were initially defined for the most studied vectors primarily found in the group of hemipteran insects, particularly aphids and related bugs (Blanc 2008; Brault *et al*. 2010; Table 2).

The first and simplest category is called 'non-circulative' transmission. In this type of virus–vector interaction, the virus is sucked up by the insect vector during feeding and can immediately attach to the cuticle lining the food and/or salivary canals within the mouthparts (stylets in the case of hemipteran insects). The virus is only transiently retained at these attachment sites and can usually be inoculated into new host plants, within a few minutes following the acquisition, upon salivation or egestion by the vector (reviewed in Ng & Falk 2006). So for non-circulative transmission, insects that solely operate short test feedings on the plant and then rapidly move on are believed to be the most efficient vectors. Long sustained feeding on an infected plant decreases efficient acquisition and transmission, if not totally abolishing it.

The second category of viral transmission is designated circulative transmission. The virus is sucked up and ingested together with plant cellular contents by the insect vectors, where it crosses the gut epithelium, diffuses into the haemolymph and reaches and accumulates into the salivary glands without replicating. After this cycle, the virus can be secreted and inoculated into new host plants together with the insect saliva (reviewed in Hogenhout *et al*. 2008a).

Finally, in the third category designated propagative transmission, the virus completes a similar cycle within the vector's body, but replicates within the gut, the salivary glands and sometime other tissues of the insect (reviewed in Hogenhout *et al*. 2008a). In circulative and propagative transmission, a 'latent period' of one to a few days is generally required to complete the viral cycle, and ulterior retention of the infectious virus in the insect vector can be long, eventually lasting until the vector dies. In both circulative and propagative transmission, insects settling on and colonizing the infected host plants are often the best vectors.

The localization of a virus within the host plant combined with the feeding habit of its vector explains in part the features of the different categories of transmission defined in Table 2. Viruses infecting all tissues of the host are readily accessible to the insect vectors and are generally acquired during initial feeding steps. In contrast, those strictly restricted to specific plant tissues will logically depend on the time needed and the frequency with which the vectors actually access and feed in such tissues. Although this assumption is trivial and certainly applies to all virus–vector couples, it has solely been thoroughly characterized with hemipteran vectors through the precise monitoring of their feeding behaviour by electrical penetration graph recording (EPG) (Fereres & Moreno 2009). Both acquisition and inoculation of non-tissue-restricted viruses (Table 2) can be achieved within seconds because they replicate and accumulate in epidermis and mesophyll, the first tissues test probed by hemipteran vectors when they alight onto a putative host plant. By contrast, viruses

Table 2. Different modes of plant virus transmission by insects with pierce-sucking mouth parts

	Circulative		Non-circulative	
Transmission modes*	Propagative	Non-propagative	Capsid strategy	Helper strategy
Acquisition time†	Minutes to hours	Minutes to hours	Seconds to hours	Seconds to hours
Retention time‡	Days to months	Days to months	Minutes to hours	Minutes to hours
Inoculation time§	Minutes to hours	Minutes to hours	Seconds to minutes	Seconds to minutes
Association with vectors¶	Internal	Internal	External	External
Replication in vectors	Yes	No	No	No
Requirement of a HC**	No	No	No	Yes

From reference Blanc (2008), with permission.
*These modes of transmission were established and are widely accepted for virus transmission by pierce-sucking insects. As discussed in the text, they sometimes also apply to other types of vector.
†The length of time required for a vector to efficiently acquire virus particles upon feeding on an infected plant.
‡The length of time during which the virus remains infectious within its vector, after acquisition.
§The length of time required for a vector to efficiently inoculate infectious virus particles to a new healthy plant.
¶Internal means that the virus enters the inner body of its vector, passing through cellular barriers. External means that the virus binds the cuticle of the vector and never passes through cellular barriers.
**A helper component (HC) is involved in cases where the virus particles do not directly recognize vectors, acting as a molecular bridge between the two.

with a phloem-restricted way of life rely on sustained phloem feeding of the vector, which occurs solely on suitable host plants, after a series of numerous test probes in superficial tissues. Their acquisition and inoculation consequently requires longer time lapses usually in the order of hours to days (Table 2).

The viral molecules involved in the specific recognition of (and interaction with) the vectors are partly elucidated (Ziegler-Graff & Brault 2008). They are generally capsid and/or envelope proteins, or other non-structural proteins forming a molecular bridge between virus particles and insect mouthparts or gut. In contrast, the counterpart receptor molecules within the vector are totally unknown and certainly represent a major challenge in the field for the next coming years (Blanc, Uzest & Drucker 2011), although beyond the scope of the present review.

Effect of the virus on plant–insect interactions

PLANT VIRUSES DIRECTLY CHANGE THE PHYSIOLOGY AND BEHAVIOUR OF INSECT VECTORS

Propagatively transmitted viruses

While infecting organs and replicating within the vector body, propagative viruses might directly affect life traits of their insect vectors (Hogenhout *et al*. 2008a). Propagative transmission is the exact equivalent of the transmission of arboviruses infecting animals (Blanc 2004). In fact, some authors have suggested that propagative plant viruses originate from insect viruses, which have secondarily acquired the capacity to also infect host plants (Jeger, Madden & van den Bosch 2009; Roossinck 2011). In numerous arbovirus–vector couples, the virus is believed to directly impact on several components of the vector fitness such as longevity, growth rate and reproduction (reviewed in Kuno & Chang 2005), as well as on feeding behaviours facilitating viral transmission (for example see Platt *et al*. 1997), or other references cited in Stafford, Walker & Ullman (2011).

Experimental data supporting these possibilities are rare for plant viruses where propagative transmission involves only a few genera. Most changes in life-history traits of insect vectors feeding on infected plants are usually attributed to virus-induced modification of the host plant (see next section), rather than to a direct modification of the vector by the ingested viruses. However, propagative viruses of plants do infect their vectors, likely affecting them at least in some instances (Hogenhout *et al*. 2008a,b; Ammar el *et al*. 2009). Sinisterra and collaborators (Sinisterra *et al*. 2005) demonstrated that *tomato yellow leaf curl virus* (TYLCV) expresses some of its genes into cells of its whitefly vectors (*Bemisia tabaci*, biotype B), whereas another member of the genus *begomovirus*, *tomato mottle virus* (ToMoV) does not. The authors suggested that, although closely related and transmitted by the same vector, these two viral species are transmitted in different ways: TYLCV is transmitted via propagative means, while ToMoV is transmitted via circulative means. The former decreases vector fitness and the latter induces no detectable deleterious effects.

Concerning the manipulation of the vector's feeding behaviour, a remarkable recent study evidenced a direct viral effect on thrips vectors infected by *tomato spotted wilt virus* (TSWV) (Stafford, Walker & Ullman 2011). TSWV-infected male thrips increased non-cell destructive feeding behaviours associated with salivation, likely ameliorating the chances of viral infection at inoculation sites, thereby enhancing the overall efficiency of transmission.

Circulatively transmitted viruses

Like the begomovirus ToMoV mentioned above, many plant viruses are circulatively transmitted and do not replicate

during their cycling within the vector body. The best-described examples of this type of transmission correspond to members of the family *Luteoviridae*, transmitted by aphids (Gray & Gildow 2003), where virus particles go across cells enclosed into membrane vesicles with no contact with the cell cytoplasm (Brault, Herrbach & Reinbold 2007). Most member species of the families *Geminiviridae* and *Nanoviridae* are commonly assumed to be transmitted by whiteflies, leafhoppers or aphids in a similar manner (Hogenhout *et al*. 2008a), although a clear description of the form under which the virus actually circulates inside the insect is still lacking. If circulative transmission proves to be exactly as it is described in the current literature, there is only little ground for the virus to directly impact on the physiology of its vector. However, because interactions between virus and specific membrane-associated receptors at the gut and salivary gland levels are strongly suspected (Brault *et al*. 2010), and because the presence of the virus might very well be detected by the immune system of the insect (Luan *et al*. 2011), its putative direct influence on fitness and behaviour of the vector cannot be excluded.

Non-circulatively transmitted viruses

Finally, in the most widely spread non-circulative transmission, plant viruses are retained specifically at the surface of the cuticle lining the inner food and/or salivary canals of the insect mouthparts (Martin *et al*. 1997; Powell 2005; Uzest *et al*. 2007, 2010), or lining the lumen of the foregut (Chen *et al*. 2011). Only the viral particles retained at these sites can rapidly be released and inoculated into new host plant, and non-retained and ingested excess virions are always considered lost. Further interactions between these ingested virions (and viral proteins) and insect molecules all along the gut transit could theoretically induce a response by the insect vector. However, because these putative more intricate interactions with the vector are not necessary for the success of non-circulative transmission, they have never been studied nor envisaged to play a role at all, albeit perhaps mistakenly.

VIRUS USES THE HOST PLANT TO INDIRECTLY MANIPULATE INSECT VECTORS

The fact that virus-infected plants can be better hosts than healthy ones for herbivorous insects was noted decades ago and has recently been reviewed (Bosque-Perez & Eigenbrode 2011; Malmstrom, Melcher & Bosque-Perez 2011). Viral infection can modulate the main plant defence pathways (Ziebell *et al*. 2012), the sap composition in amino acids (Ajayi & Dewar 1982) and the emission of volatile organic compounds (VOC) (Ponzio *et al*., this issue), potentially favouring or disabling the insect attraction, growth, reproduction and thus colonization of the host plant (Mauck, De Moraes & Mescher 2010a,b; de Vos & Jander 2010). Secondary metabolites released as VOC (see references above) are a major factor attracting or repelling herbivorous insects, but virus-induced symptoms in plants also provide visual cues to herbivorous insects, which can be attracted by specific colour (Ajayi & Dewar 1983).

With regard to vector transmission, the question of interest is whether these virus-induced changes in plants are adaptive and modify the plant–insect interplay in a way favouring enhanced virus transmission. One convincing hint is the evidence that virus-induced changes in vector behaviour specifically match viral mode of transmission (reviewed in Stafford, Walker & Ullman 2012). Briefly, a virus transmitted in a propagative or circulative way should attract and arrest insect vectors, favouring settlement, reproduction, colony formation and perhaps late production of 'migrators' to ensure maximum dissemination. A non-circulatively transmitted virus, in contrast, should have no effect or should attract but then repel vectors, because its acquisition is fast and retention extremely short. Finally, a virus transmitted by several different insect vector species might not attract vectors, while a virus transmitted by one sole-specific insect should develop a more intimate relationship. An increasing number of experimental studies are supporting the existence of all these phenomena (reviewed in Eigenbrode *et al*. 2002; Blanc & Drucker 2011; Bosque-Perez & Eigenbrode 2011; Malmstrom, Melcher & Bosque-Perez 2011; Stafford, Walker & Ullman 2012), and viruses seem to have finely tuned processes acting on all possible aspects of plant properties: size, shape, colour, odour and taste, for efficiently manipulating their vectors.

The virus-induced changes in vector behaviour and/or life traits likely have consequences for the virus epidemiology. Theoretical models have compared the influence of the transmission modes described above on disease incidence or spread. While, among other predictions, these models suggested more efficient spread for viruses interacting with their vector for longer periods (Madden, Jeger & Bosch 2000; Jeger, Madden & van den Bosch 2009), it would be interesting to refine these predictions with the putative reciprocal manipulations that are being uncovered in various virus/vector associations.

A compelling example of the possible broad-scale impact of the three-way plant–insect–virus interactions can be found in the history of begomoviruses transmitted to tomato in China. The invasion and displacement of the indigenous chinese ZHJ1 biotype of the whitefly complex *Bemisia tabaci* by the invasive biotype B in the late 1990s has been explained by the fact that tomato plants infected by indigenous begomovirus *tomato yellow leaf curl China virus* (TYLCCNV) and *tobacco curly shoot virus* (TbCSV) are much better hosts for the B biotype than for the 'local' ZHJ1 (Jiu *et al*. 2007). Interestingly enough, the next invasion wave might be occurring at present. The tomato plants infected with the middle-east-originating begomovirus TYLCV (first detected in China in 2006) are increasing the fitness of another invasive whitefly biotype Q (first

reported in China in 2003), but not biotype B (Pan *et al*. 2012). Because biotype Q transmits TYLCV with the highest efficiency, the authors proposed that a mutualistic interaction between the two explains their parallel rapid expansion across tomato cultures in China over the last 5 years.

Additional trophic levels might be integrated into the picture. They concern the effect of plant-emitted VOCs on the insect predators and parasitoids of virus-vectoring insects (Jeger *et al*. 2012) or the role of bacterial insect endosymbionts on the plant–insect or insect–virus interactions. These are addressed in other contributions of this special issue (Ponzio *et al*. and Giron *et al*., respectively). Bacteria of the genera *Buchnera* in aphids (van den Heuvel, Verbeek & van der Wilk 1994), and *Wolbachia* and *Hamiltonella* in whiteflies (Gottlieb *et al*. 2010), have been, respectively, proposed to assist the transmission of luteoviruses and geminiviruses, through the virus-protective action of chaperon GroEL-like proteins supposed to be secreted by the bacteria into the insect haemolymph. However, these results remain controversial. A recent study has shown that *Buchnera* GroEL is not secreted in significant amounts by the bacterial cells, seriously questioning the general idea of the direct involvement of insect symbionts into the circulative transmission of plant viruses (Bouvaine, Boonham & Douglas 2011).

Altogether, the above-mentioned interactions can participate in shaping the species communities within agroecosystems (Malmstrom, Melcher & Bosque-Perez 2011). Insects can vector viruses affecting the fitness of plants and their competitiveness in a given environment. In turn, the impact of viruses on insect vectors, either direct or through virus-induced modifications of plant properties, is enhancing or disabling the performance of insect species, their dispersal behaviour, their density in communities and the predation pressure they impose on various plant hosts. One clearly missing area in this field of research is the impact that insect vectors could have (beyond simple spreading among hosts) on the evolution and on the phenotypic expression of viruses. Based on very recent data from our laboratory and others, the two next sections speculate on how differential virus evolution could be driven by different vector species and on the viral life cycle switch that could be induced by the presence of vectors on the host plant.

Insect behaviour impacts on population genetics and evolution of plant virus

This section argues for a putative effect of the vector feeding behaviour on the potential for evolution and adaptation in viruses. During transmission, only few virus particles are taken up by vectors, which thus induce repeated viral population bottlenecks. The size of these bottlenecks can affect the fitness in the viral population, and, interestingly, different vector species with specific feeding habits could impose bottlenecks of variable sizes. Whether and how viruses have adapted to alleviate the effect of such demographic fluctuations is an interesting and mostly overlooked question.

STRUCTURE OF WITHIN-PLANT VIRAL POPULATION AND INSECT FEEDING BEHAVIOUR

Connections between viral location in plants tissues, feeding behaviour of vectors and virus transmission have been widely recognized for decades (see Virus-vector interactions) and recently reviewed (Stafford, Walker & Ullman 2012). Yet we believe aspects important for the outcome of virus transmission at a larger space time-scale have been overlooked. The genetic structure of the virus population occupying a territory within a host plant is of prime importance in that sense (Blanc, Uzest & Drucker 2011). Indeed, vectors take up a tiny fraction of the viral population, but the viral genetic information they actually sample and recurrently transmit is instrumental in virus evolution. Within hosts, the virus population can split and diverge into isolated subpopulations: some viral individuals remain 'inert' at initial infection sites in old leaves, while others continuously replicate in newly formed tissues. Keeping this in mind, the impact of feeding habits of the vectors visiting the plant needs renewed attention. Key parameters for research are not only the architecture of the plant, but also its growth/development and the concomitant population dynamics and genetic structuring of the invading virus population.

In asexual populations with a finite size, demographic fluctuations and particularly the occurrence of strong population bottlenecks are theoretically predicted to increase stochastic changes in allele frequency (genetic drift), due to random sampling in one population (or generation) to initiate the next one. These random changes are associated with the accumulation of mutations in genomes (most mutations being deleterious), a process called Müller's ratchet (Muller 1964). Empirical demonstration of this phenomenon was obtained through experimental evolution of the bacteriophage Φ6 (Chao 1990), followed by studies on viruses of animals (Duarte *et al*. 1992) and plants (de la Iglesia & Elena 2007). These virus models consistently showed that when several successive bottlenecks are imposed on viral populations, with no or negligible recombination, mutations indeed accumulate engendering a dramatic and rapid decrease in fitness of several orders of magnitude. Two distinct processes can interfere with the viral fitness decrease induced by Müller's ratchet. First, genetic exchanges such as recombination can both restore non-mutated genomes from differentially mutated parents and/or accelerate the purging of deleterious mutations by cumulating them on defective genomes rapidly eliminated from the population (Felsenstein 1974). Second, functional complementation – a functional gene product encoded by one genome assists related genomes deficient in the same gene – can compensate the negative effects of deleterious mutations and thus delay their purging from the viral

population. This action of recombination and complementation has been studied experimentally with the bacteriophage Φ6 and demonstrated to be dependent on the number of coexisting genomic variants within individual infected cells of the host (Froissart *et al.* 2004). Recombination proves extremely frequent in numerous DNA and RNA viruses of plants (Froissart *et al.* 2005; Urbanowicz *et al.* 2005; Martin *et al.* 2011), and complementation is a rampant phenomenon in all viruses described thus far (see for example Moreno *et al.* 1997; Aaskov *et al.* 2006; Simon *et al.* 2006; Paolucci *et al.* 2011). However, one limitation of their relevance might be the often low diversity of genomes coreplicating at a given host location, a parameter intimately linked to population bottlenecks.

Within the last decade, viral population dynamics and genetics have been analysed at the scale of a single host plant (Garcia-Arenal & Fraile 2010; Elena *et al.* 2011). Very severe demographic bottlenecks have first been demonstrated during invasion of wheat plants by *Wheat streak virus* (French & Stenger 2003), where the number of viral genomes initiating the infection of new tillers has been estimated to be as small as a few genome units. Similar figures were then reported for *tobacco mosaic virus* (TMV) systemically infecting a tobacco leaf from the initially inoculated one (Sacristan *et al.* 2003). These demographic bottlenecks can isolate small fractions of the diversity of viral populations in individual leaves, inducing a differentiation of subpopulations at various plant locations due to strong genetic drift. Although not formally quantified, this phenomenon has also been shown with *cucumber mosaic virus* (CMV, Li & Roossinck 2004) and *plum pox virus* (PPV, Jridi *et al.* 2006), suggesting that it is widespread among plant RNA viruses.

In addition to bottlenecks, another enigmatic mechanism increasing the patchwork distribution of the viral genetic diversity within leaves is the lack of cell superinfection, a phenomenon observed for all plant viruses tested thus far. Unknown molecular interactions inhibit the secondary infection of cells (Folimonova 2012) and leaf areas that are initially invaded by one genomic variant become totally refractory to super infection by any other closely related variant (Dietrich & Maiss 2003; Takahashi *et al.* 2007). An illustration of such mutual exclusion is presented in Fig. 2, showing a leaf systemically infected by two identical *turnip mosaic virus* (TuMV) clones, labelled with the red fluorescent protein (RFP) and green fluorescent protein (GFP), respectively. The patchwork distribution of green and red fluorescence demonstrates a spatial segregation of the two clones, typically alike that previously published for other viruses in the references cited above.

Aphids are important vectors of these viruses, and the number of viral genomes they transmit has been estimated between one and a few units for potyviruses (Moury, Fabre & Senoussi 2007), cucumoviruses (Ali *et al.* 2006; Betancourt *et al.* 2008) and caulimoviruses (our own unpublished results). So, although vector transmission indeed induces dramatic bottlenecks in viral population,

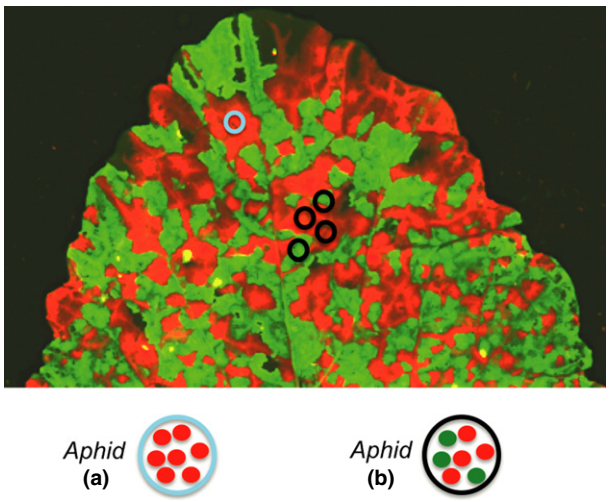

Fig. 2. Spatial segregation of *turnip mosaic virus* and relation with different feeding behaviours of aphid vectors. A turnip host plant was coinoculated by two TuMV variants, each encoding and thus producing a different fluorescent protein (GFP in green and RFP in red). Spatial segregation is particularly easy to visualize here through the mutual exclusion of the two colours. Each clone can be observed to separately infect leaf cells, yielding a patchwork of infected regions with a single fluorescence. The circles on the leaf represent probes of two different aphids. Aphid A is making very few test probes before departing and transporting only one of the two variants, whereas aphid B is making several probes potentially inducing the uptake of the two variants.

cotransmission of more than one viral genome by a single aphid is possible and was evidenced four decades ago for potyviruses and caulimoviruses (Govier & Kassanis 1974; Lung & Pirone 1974). In Fig. 2, we illustrate two different outcomes of virus uptake by insect vectors in a heterogeneously distributed viral population. Numerous plant viruses can be transmitted by more than one vector species, and it is common that an aphid species A conducts few test probes on a host, whereas a species B conducts many more test probes (Fereres & Moreno 2009; Stafford, Walker & Ullman 2012). For equal numbers of transmitted viral genome, A is likely to induce a genetic bottleneck dramatically more severe than B. If one pursues this reasoning, the evolution of the same virus species can differ depending on the vector species that is dominating in a given environment and at a given time, and this phenomenon can be modulated by different densities of the vector populations (Escriu, Fraile & Garcia-Arenal 2003). The possible impact of vector feeding behaviour on virus population genetics could be easily tested by an estimation of the size of the genetic bottlenecks induced by single aphids after one or several intracellular punctures on leaves infected by distinct viral genetic variants as in Fig. 2. Further, experimental evolution of two viral lines serially transmitted by aphid vectors, one line transmitted via a single intracellular puncture at each round of transmission and the other through several probes in different cells, could test for a differential evolution of viral fitness under the two regimes.

Whether viruses have adapted specific mechanisms to alleviate the problem of the repeated genetic bottlenecks induced upon insect transmission is unknown. The role of the so-called helper components, which are viral proteins linking the virus particles to their receptors inside the vectors, is the only putative example that has been discussed in this way (Pirone & Blanc 1996; Froissart, Michalakis & Blanc 2002). These molecules are capable of specific attachment to the receptors in the vector and can secondarily link virus particles, likely facilitating the complementation between viral genomes for transmission (Fig. 3). Noticeably, if a virus distributed within the plant as in Fig. 2 uses a helper component for its interaction with the vector, a functional helper encoded by a green genome could assist the transmission of a deficient red genome during successive intracellular punctures at different locations (aphid B). In contrast, a virus species that does not produce a helper component, and directly binds the vector receptor through its capsid protein, will not allow complementation even upon multiple punctures at different locations by the vector B.

In summary, it is obvious that the viral genetic diversity available to the vector depends on its distribution within the host plant and on the way the insect vector is actually foraging on it. The existence of genetically heterogeneous viral subpopulations, and of distinct feeding behaviours in different vector species, have both received considerable support. However, the direct experimental test of a diverging evolution in viral lines transmitted by distinctly behaving vectors is still lacking.

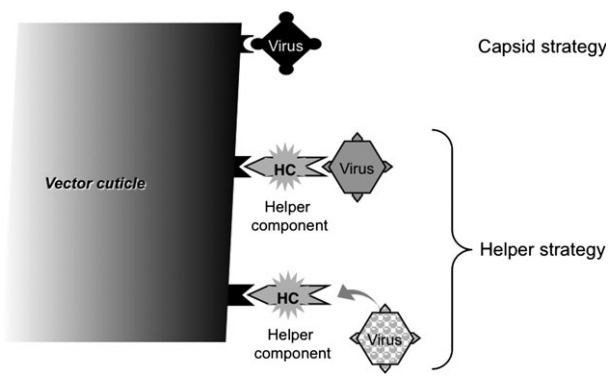

Fig. 3. Two molecular strategies for virus–vector interaction in non-circulative transmission of plant viruses. Both strategies allow the retention of virus particles in the vector mouthparts or foregut on putative receptors located at the surface of the cuticular lining. In the capsid strategy, a motif of the coat protein is able to directly bind to the vector's receptor. In the helper strategy, virus–vector binding is mediated by a virus-encoded non-structural protein, the helper component (HC), which creates a reversible 'molecular bridge' between the two. HC can be acquired alone, prior to virion, and thereby allow HC-transcomplementation. In this case, a HC encoded by a genome X (for instance that encapsidated in the gray virion) can subsequently assist the transmission of a genome Y of the same population, encapsidated in the dotted virion. This possible sequential acquisition of HC and virion is symbolized by the arrow. It has been demonstrated experimentally that HC and virion can be acquired in different infected cells or even different hosts. From (Froissart, Michalakis & Blanc 2002) with permission.

DYNAMICS OF WITHIN-PLANT VIRAL POPULATION AND INSECT FEEDING BEHAVIOUR

An extension of the previous section is that the relationship between the structure of viral populations and the feeding habits of vectors should also be considered in the context of spatio-temporal dynamics. Whether vectors attack the plants early or late in infection, and whether they prefer feeding in young developing or in fully matured leaves, the viral population they 'meet' is certainly significantly different.

In a recent study, we have demonstrated that the titre of CaMV within the phloem sap of infected turnips is not constant (Gutierrez *et al.* 2012). It increases sharply from early infection until full systemic plant invasion and then later decreases back to initial viral load before plant flowering and senescence. Because the phloem sap is the medium transporting the virus long distance, from leaf to leaf and into differentiating young newly formed leaves, we hypothesized that it contains a variable inoculum dose that could affect the size of bottlenecks along the progression of infection over time. In accordance with this hypothesis, we found that the bottleneck size at leaf entry varies from a few viral genomes for early-infected leaves, to hundreds of viral genomes for intermediate leaves, and back to few genome units for leaves appearing just before flowering and senescence. This is consistent with an earlier study on the same virus (Gutierrez *et al.* 2010), demonstrating that the number of viral genomes initiating infection of individual cells within each leaf (multiplicity of cellular infection; MOI) followed a similar pattern, with a MOI around 1 or 2 in early- and late-developing leaves and peaking at around 15 in intermediate leaves (Fig. 4).

This latter observation suggests that the CaMV variants appearing and circulating within a host plant can be accessible in individual cells either as isolated clones or as mixture of clones, depending on the specific leaves visited by the vector. Here again, if the vector can acquire and transmit more than one viral particle, the viral MOI in the visited plant cells might be a key parameter for the associated genetic bottleneck (Blanc, Uzest & Drucker 2011). It is noticeable (Fig. 4 and associated reference) that leaves with cells infected at different MOIs coexist on infected plants. Hence, the preference of aphid vector species for young or old leaves, for high-light-exposed or lower shaded leaves, or their migration very early or later in the crop season might change the transmission bottlenecks in viral populations and hence differentially affect their evolution. Whether the reported dynamic variations of bottlenecks and MOI during plant colonization by CaMV can be extrapolated at least to some other plant virus species will require further investigations, because both parameters have been estimated at a more restricted time-scale for TMV (Sacristan *et al.* 2003; Gonzalez-Jara *et al.* 2009)

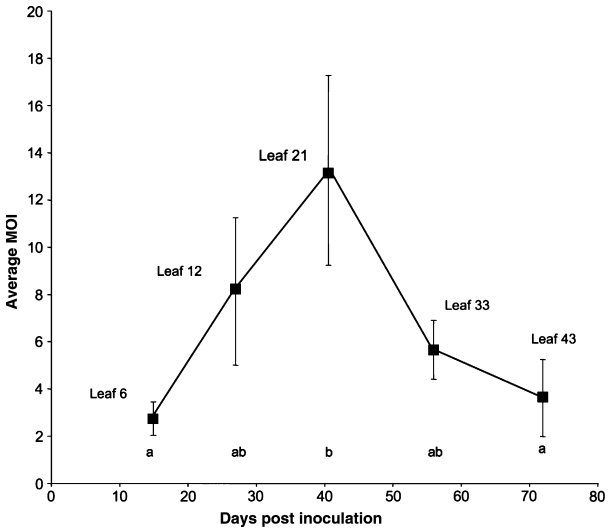

Fig. 4. Dynamics of the multiplicity of infection of cells (cellular MOI) by *cauliflower mosaic virus* in turnip host plants. Each point represents the average estimate of the MOI in individual cells of a systemically infected leaf. Six leaves successively appearing on the infected plants have been similarly analysed, demonstrating a dynamic pattern of MOI over time. Bars represent standard errors. Different letters between two estimates indicate significant differences ($P < 0{\cdot}05$). Note that when leaf 21 appears, leaves 6 and 12 are still present on the plant, thus leaves infected at different MOI coexist at a given time point. From (Gutierrez *et al.* 2010), with permission.

and soilborne wheat mosaic virus (SbWMV, Miyashita & Kishino 2010).

The influence of MOI on the size of bottlenecks during vector transmission is an important question, which could be addressed by estimating the number of CaMV genomes transmitted by aphids fed on leaves infected at high and low MOI. Furthermore, experimental evolution could monitor changes of the CaMV fitness in parallel lines serially transmitted by aphids, either from leaves with high MOI or from leaves with low MOI.

Such experiments would likely demonstrate the possibility that, depending on how spatio-temporal dynamics of within host viral populations and feeding patterns of vectors actually match, the evolution of viruses could be differentially driven by different vector species.

Plant–insect molecular dialogue can change virus behaviour

In Effect of the virus on plant–insect interactions, we have shown how viruses can impact the behaviour and life cycle of their insect vectors, in a way that ultimately enhances the chances of transmission. In this section, we discuss an unforeseen complementary scenario, where the vector provokes changes in the virus infection cycle, which rapidly switches to a 'transmission mode'. In fact, we have recently discovered that a virus can literally perceive the vector feeding on the plant and immediately react and produce transmission specialized forms within the infected plant cells (Martinière *et al.* 2013).

When a virus is introduced in a host plant, it first replicates within the inoculated cell and then moves from cell to cell through plasmodesmata until it reaches the vascular system allowing long-distance movement and systemic plant colonization. When feeding on a plant, an insect vector can theoretically take up the virus at any step of the infection cycle, and it is always implicitly assumed that virus 'material' is taken up randomly by the vector, together with the ingested plant cell content or sap. So the current view is that the virus interacts with the host plant to replicate and progress systemically and, somewhere along this process, a vector comes and feeds on the infected plant, eventually acquiring the virus.

Interestingly enough, some other types of pathogens also transmitted by insect vectors no longer support this simple view. Indeed, in parasitic bacteria and metazoans, specific morphs specialized in transmission have been repeatedly reported (reviewed in Matthews 2011). This implies that the allocation of resources by the pathogen, either to colonization of the host or to transmission from host to host, is regulated during its life cycle. It can even be conditioned by stimuli from the environment of the host, including stimuli coming from the vector itself (Matthews 2011). Until recently, there were no reports of distinct morphs of plant viruses specialized in host-to-host transmission. However, recent observations on the transmission of CaMV by aphids have drastically changed this view (Fig. 5 and Martinière *et al.* 2013).

In infected plant cells in close vicinity to aphid stylets, this virus immediately and reversibly transmutes into a form that greatly enhances its acquisition. The process starts with the formation of the well-known inclusion body [transmission body (TB)], specialized in the regulation of virus acquisition by the aphid vector, in each infected plant cell (Khelifa *et al.* 2007; Martinière *et al.* 2009). But the insertion of the aphid stylets in between and/or across these cells appears to trigger a spectacular change of the TB, which is massively loaded with soluble tubulin within seconds, prior to total disruption and relocation of its viral content (virus particle and helper component P2) onto the microtubule network, all over the cell cytoplasm. This greatly enhances the accessibility of the virus, which only then is efficiently acquired and transmitted (Martinière *et al.* 2013). The actual aphid-associated signal for this TB transformation remains elusive but the authors suggested a mechanical stress due to stylet's activity, a chemical signal from a compound of the aphid saliva, or both. An observation that is highly relevant for the present review is that other unrelated stresses such as wounding, heat shock or high CO_2 ppm in the atmosphere could also induce partial or complete TB changes, thereby predisposing the infected plants to enhanced vector transmission success. This non-aphid induction of the TB changes is likely due to partial overlaps of the plant signalling pathways triggered by the aphid and by some other unrelated stresses, which could

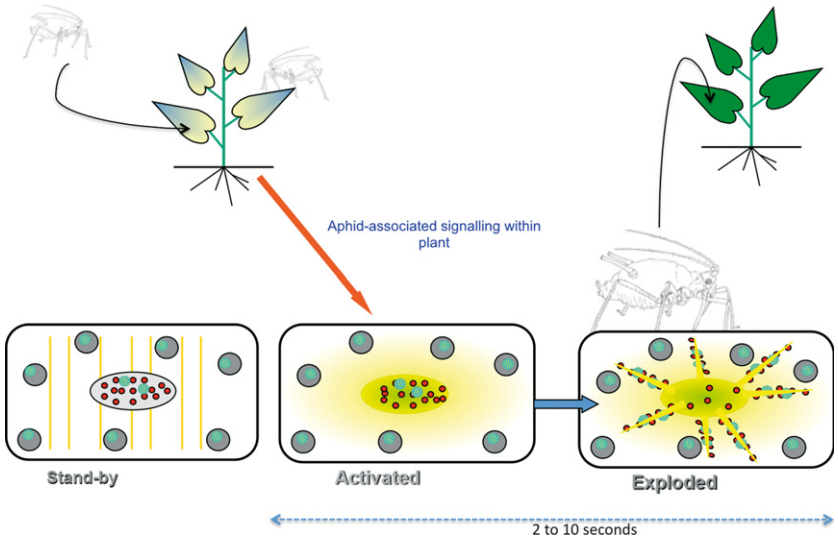

Fig. 5. CaMV produces structures specialized for aphid transmission in infected plant cells. The whole infection cycle of CaMV is schematized chronologically from left to right. First an aphid vector inoculates the virus into a host plant, which becomes systemically infected after several days. In infected plant cells, the viral molecules required for vector transmission, the helper protein P2 (red dots) and the mature virus particles (yellow/bleu icosahedra) accumulate in a specific inclusion body designated the 'standby' transmission body (TB). When an aphid vector feeds on the infected plants, it triggers an immediate response of the TB, which is massively loaded with soluble tubulin (yellow) and then named the 'activated' TB. The activated TB subsequently disrupts and its components are released and distributed onto the cellular microtubule network (yellow), thus increasing their accessibility to the aphid vector, ensuring efficient acquisition and transmission. The time lapse between the aphid triggering signal and the virus acquisition by aphids from the 'disrupted' TB is in the order of few seconds (light blue dotted line). This figure has been elaborated by S. Blanc, A. Bak, A. Zancarini A. Martinière and M. Drucker, from results published in (Martinière *et al.* 2013).

be hijacked by the virus and translated into sudden TB changes.

The inspiring possibility that some plant stresses could falsely be perceived as a vector signal by the virus has putative important ecological impacts. The effect of abiotic plant stress, and more generally of the plant environment, on virus accumulation, symptom expression and transmission has received recent attention (Xu *et al.* 2008; Borer *et al.* 2010; Cronin *et al.* 2010; Schrotenboer, Allen & Malmstrom 2011; Fu *et al.* 2012; Huang *et al.* 2012; Suntio & Makinen 2012). Plant signalling pathways and responses to various abiotic stresses are partly shared with those induced by viral infection, and the fact that they can interfere with one another is not a novel concept (see Suntio & Makinen 2012 and references therein). Nevertheless, we believe it deserves further exploration and, in particular, virtually no studies report any changes in the rate of vector transmission of a virus that could be attributed to the action of an additional biotic or abiotic stress. Two main reasons suggest that this is a major parameter to be considered in the ecology of viruses. The first is that viral accumulation is influenced by these stresses (Suntio & Makinen 2012), and the increase in viral load in infected plants could result in increased transmission. The second is inspired by the observation summarized above for CaMV, which uncovers an overlooked phenomenon: viruses can produce transmission-specific morphs in response to an insect vector 'signal' transduced by the plant, and various unrelated stresses can trigger this response, predisposing the host plant as a better viral source for vector transmission (Martinière *et al.* 2013). The investigation of the relationship between various plant stresses and the efficiency of vector transmission of infecting viruses thus appears to be an important future prospect, but it will be an enormous task to be addressed as a case-by-case study. Nevertheless, with the foreseen global climate change, a viral life trait as important as transmission is definitely worth characterizing in the context of other components of the host plant environment, whether biotic or abiotic.

Speculative prospects

Previous sections delineate a number of future research directions, largely based on recently published data. One promising area is the response of plant viruses to cues from the host plant and its environment, which could signal the presence of potential vectors and results in viral transformation into transmissible stages. Two recently published reports are pointing at the capacity of viruses to react to cues from the environment of their host plants. In the first of these reports (Dorokhov *et al.* 2012), TMV is 'reacting' to methanol vapours emitted by wounded plants and increases its accumulation even in the surrounding non-wounded hosts. Of course, TMV increased accumulation is linked to changes in the host plant that is in fact responding to methanol as an interplant signalling of an herbivore or pathogen attack. The authors interpret these TMV changes as an indirect 'side effect' of changes in the

host plant, where methanol signalling is inducing an increased intraplant communication by a better gating of plasmodesmata (opening cell-to-cell and systemic communication pipelines), which is in turn profitable to the virus spread. We simply note here that TMV is transmitted in nature exclusively via wounding (Sacristan *et al*. 2011) and that reaction to a methanol signal could also be seen as an active viral response to the perception of a wounding agent, so an opportunity for transmission in the 'neighbourhood'. Only additional investigations of the molecular details underlying this intriguing viral behaviour will distinguish between a non-specific secondary effect of the plant reaction on TMV and an active TMV response to a specific signal increasing its chances of mechanical/contact transmission. The fact that a virus can literally sense the presence of its vector feeding on the host plant, and accordingly change its behaviour within infected cells to favour transmission, has been definitely proven in CaMV (Martinière *et al*. 2013) and is summarized above. Once demonstrated that viruses can directly react to the environment of their host plants, and more specifically to their insect vectors, elucidating how diverse and of what nature the viral responses could be represents a fascinating novel research horizon.

A second novel avenue is research into some of the more extreme virus strategies that we could hypothesize for viruses to enhance their transmission. Below, we discuss the speculative hypothesis that particular types of plant viruses could conditionally kill their host plants, depending on the presence of the insect vector. No hint supporting such hypothesis is yet available in the literature related to viruses, but we feel it is an attractive scenario deserving exploration. Let us look at the life and death relationship within a virus–plant couple. Viruses are obligate parasites, which can only develop in live hosts. In that sense, they profit from the survival of the host and sometimes evolve mutualistic interactions (reviewed in Roossinck 2011). Even pathogenic viruses require hosts to live long enough to allow efficient reproduction and transmission (Froissart *et al*. 2010). The point of interest here is the 'easiest viral choice' when a vector is arriving and feeding on the infected host plant. For non-circulative viruses (see Fig. 1 and Table 2), the significant vectors are those passing rapidly on the host and moving on to the next host. So, the longer the host plant stays alive, the more it is likely to be visited by vectors and to provide transmission opportunities. In contrast, for viruses that are acquired during long feeding periods and persist as an infectious component for the vector's lifetime, different opportunities arise. The best vectors for this type of viruses are often insects colonizing the host plant for the long term. We here speculate that the virus could adopt two distinct strategies: i) promote plant survival as long as possible and compete with the insect for plant resources to allow more opportunities for acquisition and transmission and ii) allow a sufficient length of time for the vector colonies to develop and for efficient virus acquisition and then directly or indirectly force the vector to disperse. The simplest way to implement the second strategy would be to kill the host plant, thereby forcing the vector colony to disperse onto new hosts, accelerating virus dissemination. That parasites can kill their hosts when it is beneficial for them has been demonstrated and discussed in many instances (see for example Lefevre & Thomas 2008; Matthews 2011). In the case of viruses, however, the augmented symptoms that are sometimes observed when a virus-infected plant is attacked by an insect vector have not been envisaged in this way. It is always implicitly assumed that the increased deleterious effect on the plant results from an additive or synergistic negative impact of the two, rather than from a possible vector-induced change of the viral aggressiveness.

In the 'host-killing' strategy, we here speculate that some viruses and particularly circulative plant viruses can perceive the plant signals for the presence of insect vectors and switch a major aspect of their life cycle (through altered regulation of gene expression governing accumulation, tissue tropism, countering of plant defences, etc.), from preserving the host alive to killing it rapidly. We believe this hypothesis is appealing and deserves further attention, not only as an unforeseen viral 'tool' to enhance spread, but also as an important outcome of the plant–microbe–vector interaction within ecosystems.

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Conflict of interest

All authors declare no conflict of interest.

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